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Original Article

Evaluation of Cefoxitin Disk Diffusion Test for Routine Detection of Methicillin-resistant Staphylococcus Aureus

Mohammad Rahbar[1], Nooshafarin Safadel[2]

1. Department of Microbiology, Reference Laboratories of Iran, Tehran, Iran.
2. Department of Pathology, Reference laboratories of Iran, Tehran, Iran.

ABSTRACT

Background and Objectives: **Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community acquired infections. Detection of MRSA in laboratories is very important for treatment and appropriate infection control. The aim of this study was to evaluate cefoxitin disk diffusion method for detection of MRSA and comparison of this method with other conventional methods.**

Methods: **A total of 175 clinical isolates of S. aureus isolated from clinical specimens were studied. The isolates were identified by conventional laboratory methods. In this respect, E-test MIC, cefoxitin and oxacillin disk diffusion methods, and MAST ID Methicillin strips were used for detection of MRSA. All disk diffusion methods were performed as recommended by NCCL and manufacturers' guidelines.**

Results: **Using E-test MIC, 53 out of 175 strains of S. aureus were resistant to methicillin. In addition, disk diffusion method using oxacillin disk showed that 52 strains are resistant to methicillin. In this respect, 8 strains had intermediate resistance to methicillin. For cefoxitin disk diffusion method, 52 strains were resistant to methicillin. This method had a good correlation with E-test MIC method. Meanwhile, MAST ID methicillin strips detected 47 strains that were resistant to mehicillin. Sensitivity and specificity for both cefoxitin and oxacillin disk diffusion methods were 98%and 100% respectively. However cefoxitin was better than oxacillin for detecting intermediate resistant strains of S. aureus. Sensitivity and specificity for MAST ID methicillin strips were 91% and 100% respectively.**

Conclusion: **This study revealed that cefoxitin disk diffusion method is a good alternative for oxacillin disk diffusion method for detection of MRSA. This method is more reliable for identification of intermediate resistant strains of S. aureus.**

Key words: **Methicillin-resistant Staphylococcus aureus, Cefoxitin**

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Address communications to: Mohammad Rahbar, Department of Microbiology, Reference Laboratories of Iran, P.O. Box:1717-365 Tehran- IRAN
E-mail: mhhf_rz@yahoo.com

Introduction

Staphylococcus aureus has been recognized as one of the major pathogens in humans in both com

munity and hospitals (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causes of nosocomial infections. Methicillin as a semi-synthetic penicillinase-resistant penicillins was introduced in 1960 for the treatment of penicillinase producing strains of *S. aureus* and methicillin-resistant strains of *S. aureus* were identified in 1961(2). Treatment of infections caused by these strains has become problematic. Indiscriminate use of multiple antibiotics especially in developing countries, prolonged hospitalization, intravenous drug abuse, and transfer of MRSA through nose are few implicated risk factors for MRSA actuation (3).

Accurate and routine phenotypical detection of MRSA is difficult using standard disk diffusion, MIC determination, or agar breakpoint methods. This issue has been ascribed to the heterogeneous expression of methicillin resistance in many strains of *S. aureus*. Detection of *mecA* or PBP2a is therefore considered as the gold standard for exposing methicillin resistance in *S. aureus*. Few laboratories however have the technical and/or economical capacity to apply these tests to all isolates of *S. aureus* found at the microbiological laboratories. Disk diffusion methods using oxacillin is the most widely used method, but results are influenced by several factors including concentration of NaCl, temperature, inoculum, and test agent. Thus, there still remains a need for a reliable test for MRSA that can be performed easily in routine situations. Recently, the cefoxitin disk diffusion method has been proposed as an alternative method for detecting MRSA (4). Cefoxitin is a cephomycin-type antibiotic and has been described as an inducer of methicillin resistance by producing the PBP2a. No specific incubation temperature is required and the test is less affected by hyper-production of penicillinase (5). In this context, the use of cefoxitin rather than oxacillin for disk diffusion test has been advocated. Therefore, the present study compared the performance of disk diffusion tests for cefoxitin, oxacillin, and other two methods including methicillin strips and E-test for detection of MRSA.

Materials and Methods

The cross-sectional protocol of this study was carried out in Milad hospital (Tehran) from 15 April 2005 to 15 October 2005 to detect MRSA. A total of 175 strains of *S .aureus* isolated from varieties of clinical specimens including urine, blood, wound, and tracheal tube aspirates. All isolates identified by conventional microbiological methods including colony morphology, Gram stain, catalase test, slide cogulase test, tube coagulase tests, and DNAase test (6). Methicillin resistance susceptibility testing for detection of MRSA was determined using cefeoxitin 30 microgram (Hi media, India) and oxacillin 1 microgram (Mast diagnostic group, UK) as recommended by NCCLS (7). We evaluated methicillin strips for detecting MRSA (methicillin strips are filter paper strips 75 mm by 6mm, printed methicillin). Each strip impregnated with 25 µg of methicillin. A clearly defined zone of inhibition around the strip of any size interpreted as sensitive and no or little zone of inhibition around strip interpreted as resistant as recommended by manufacturer. Susceptibility testing to the other antibiotics was performed by disk diffusion method as recommended by NCCLS (6).

Finally E-test method (AB Biodisk, Solna, Sweden) was used to determine MIC as recommended by manufacturer (Briefly, using Muller-Hinton agar with 2% NaCl and an inoculum density equivalent to 0.5 Mc Farland standards, application of inoculum with a swab and incubation at 35 °C for 24 hours). We used this method as gold standard and other methods were compared with E-test MIC. The other antibiotics used for susceptibility testing were penicillin (10 IU), erythromycin (15 µg), clindamycin (2 µg), linozolide (30 µg), mupirocin (5 µg), vancomycin (30 µg), and trimethoprim- sulfamethoxazole (1.25/23.75 µg). All antibiotic disks were provided by Mast diagnostic group (UK). Interpretive criteria (in mm) for oxacillin disk diffusion tests regarding *S. aureus* were ≥ 13 mm as susceptible, 11-12 mm as intermediate, and ≤10 mm as resistant. Interpretive criteria (in mm) for *S. aureus*

using cefoxitin disk were ≥ 20 mm as susceptible and ≤19 mm as resistant. *Stphylococcus aureus* ATCC25923 and ATCC 29213 were used as quality control strains.

Results

Using E-test MIC, it was found out that 53 of 175 strains of *S. aureus* were resistant to methicillin. Disk diffusion method using oxacillin disk showed 52 strains resistant to methicillin. In this method, 8 strains had intermediate resistance to methicillin. For disk diffusion method using cefoxitin, 52 strains were resistant to methicillin(Table 1). This method had a good correlation with E-test MIC method. MAST ID methicillin strips showed 47 strains of *S. aureus* as resistant to mehicillin. Sensitivity and specificity for both cefoxitin and oxacillin disk diffusion methods were 98%and 100% respectively. However, cefoxitin was better than oxacillin to detect intermediate resistant strains of *S. aureus*. Sensitivity and specificity for MAST ID methicillin strips was 91% and 100% respectively. All isolates were susceptible to linozolid and vancomycin and 1.6% were resistant to mupirocin and 28.5% of isolates were resistant to trimethoprim- sulfamethoxazole.

Table 1. Evaluation of different methods for detection of MRSA

Method	Resistant	intermediate	Susceptible
E-test MIC	53/175	-	122/175
Oxacillin disk	52/175	8	115/175
Cefoxitin disk	52/175	-	123/175
Methicillin Strip	47/175	-	128 /175

Discussion

Oxacillin resistance in *S. aureus* is caused by expression of penicillin-binding protein 2a (PBP2a) encoded by the *mecA* gene complex. Laboratory methods have been developed to enhance the expression of methicillin resistance in staphylococci by modification of test condition, including supplementation of media with NaCl and prolonging the incubation period. Phenotypic methods for detecting MRSA strains are not usually correctly identified. Problems in detection of MRSA may be caused by low-level expression of oxacillin resistance in some strains of *S. aureus* (8-10). Cefoxitin is considered to be a better predicator than oxacillin for detecting oxacillin heteroresistance because it is stronger for PBP2a detection. In addition, it has high affinity for staphylococcal PBP4 and previous experiments have shown a relationship between PBP2, PBP4 and methicllin resistance. Many studies have reported that using cefoxitin disk has a high sensitivity and specificity (11). In a study by Velsco et al, different methods for detection of MRSA were evaluated. In their study, cefoxitin disks had 100% sensitivity for MRSA and showed negative and positive predictive values of 100% and 98% respectively. They also concluded that in the absence of availability of molecular biology techniques, the cefoxitin disk was the best predictor for methicillin resistance in *S. aureus* among other available techniques (12). In addition, Boutiba-Ben et al reported 96.5% sensitivity and 99.1% specificity for cefoxitin in detecting MRSA (8). In a study by Skov and co-workers, the cefoxitin method was excellent with 100% sensitivity and 99% specificity (4). Meanwhile, Felten et al showed a 100% sensitivity and specificity for cefoxitin. In our study using cefoxitin for detection of MRSA, 98% sensitivity and 100% specificity was obtained that was similar to results of other previous studies (Table 2).

Table 2. Comparison of sensitivity and specificity of oxacillin and cefoxitin for detection of MRSA in different studies

Study	Oxacillin		Cefoxitin	
	Sensitivity(%)	Specificity(%)	Sensitivity (%)	Specificity (%)
Boubaker BB et al	90.4	99.1	96.5%	100%
Feltn e al	96.4%	100	100	100
Skov et al	78	99	100	99
Present study	98	100	98	100

Errors in determining oxacillin resistance may have serious adverse clinical consequences. False negative susceptibility results may lead to treatment failure and the spread of MRSA, especially if appropriate infection control measures are not applied. Conversely, false-resistance results may increase health care cost following unnecessary isolation precautions and may lead to overuse of glycopeptides such as vancomycin (8).

Detecting the *mecA* gene (or PBP2a) is recognized to be the most accurate and gold standard method for detection of MRSA. However, use of PCR assay is generally limited to referral laboratories especially in developing countries and neither method is used widely for routine methicillin susceptibility tests in diagnostic laboratories (13-14). This and other studies revealed that cefoxitin disk susceptibility test appear to be a useful procedure in that it is easy to perform in routine laboratories and has greater accuracy than oxacillin disk diffusion test. The cefoxitin disk diffusion test has the potential of wider use in diagnostic microbiology laboratories.

Conclusion

this study reveals that cefoxitin disk susceptibility test appear to be a useful procedure for detection of MRSA. This method is easy to perform in routine laboratories and has greater accuracy than oxacillin disk diffusion tests.

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